VPCPVTTD (SEQ ID NO:65), 433, VLMAMSQI (SEQ ID NO:66), 445, TDCSLPMI (SEQ ID NO:67), 449, LPMIWAQKTNTPA (amino acids 3-15 of SEQ ID NO:68), 472, TFAGGVHPAI (SEQ ID NO:69), 472, TFAGGVHP (amino acids 1-8 of SEQ ID NO:69), 481, IALREYRKKMDIPAKL (SEQ ID NO:70), 484, REYRKKMD (amino acids 4-11 of SEQ ID NO:70).

13. (twice amended) The method of claim 12 wherein the peptide is labelled with a compound selected from the group consisting of dyes, fluorescent labels, chemiluminescent labels, enzymes, and radioactive labels.

14. The method of claim 12 wherein the peptides are immobilized onto a substrate.

15. (amended) The method of claim 14 further comprising detecting autoantibodies in [the] a patient sample by reacting the patient sample with the immobilized peptides.

16. (twice amended) The method of claim 15 further comprising predicting the prognosis of the patient based on the reactivity of the patient sample with different peptides.

Remarks

The specification has been amended to recite the sequence listing ID numbers, as requested by the Examiner. This amendment has been updated from the Amendment mailed November 27, 1996 (a copy of which was enclosed with the Amendment filed January 22, 2202, which provides the basis for each amendment), to comply with the current sequence listing rules and rules for submission of marked and clean copies of the specification.

In response to the Office Action mailed December 2, 1996, rejecting claims 15 and 16

under 35 U.S.C. 112, second paragraph, as indefinite, claims 15 and 16 have been amended to correct antecedent basis, as suggested by the examiner.

In response to the statement that the correction in the amino acid sequence made in the Preliminary Amendment dated September 22, 1993, should not be entered on the basis that the changes were not just correcting typographical errors, it should be noted that this objection was raised for the first time some four amendments to the specification and claims later. However, the amendments are clearly to correct typographical errors since the sequences are derived from the sequence of proteins that were all known and in the prior art before the filing of this application. In particular, the amendments at: page 6, line 6, changing the O in "QVMTPOGRGTVA" to Q (a Sm B/B' peptide); page 14, line 6, deleting the first K in "KKTKIRRSPSKPL" (La/SSB); page 18, line 21, replacing the O in "ALAVTKYKORNGWSHKDLLRSH" with Q (Ro/SSA); page 18, line 35, replacing the O in "ASMNORVLGS" with a Q (Ro/SSA); page 18, line 44, replacing the N in "NTKYITKGWKEVHEL" with IV (Ro/SSA); page 19, line 22, inserting a K after the first G in "HMVYSKRSGPRGY" (70 kD nRNP); page 19, line 23, deleting the first V in "YVKHADGKKIDGRRVL" (70 kD nRNP); page 19, line 28, replacing O in "LVSRSLKMRGOAF" in Q (A peptide of nRNP); page 19, line 29, replacing O in "ALOGFKIT" with a Q (A peptide of nRNP); and page 20, line 5, replacing the O in "AFOOGKIPP" with a Q (C peptide of nRNP).

As noted in the specification, the sequences on which the peptides are based were

published as of the date of filing: Ro/SSA is described in the parent application (referenced at page 4, lines 29-32), now U.S. Patent No. 5,637,454 which references in Example 1, the predicted amino acid sequence for the human 60 kD Ro/SSA gene product, reported by Deutscher, et al., Proc. Natl. Acad. Sci, 85:9479-9483 (1988) and Ben-Chetrit, et al., J. Clin. Invest. 83:1284-1292 (1989). La/SSB is described by Chambers, et al., J. Biol. Chem. 263:18043 (1988) and Sturgess, et al., J. Immunol. 140:3212 (1988) (page 10, lines 17-20); Sm B/B' is described in Rokeach, et al., J. Biol. Chem. 264:5024 (1989), Sharpe, et al., FEB Lett. 250:585 (1989), and Schmauss, et al., Nucleic Acids Res. 17:1733 (1989) (page 21, lines 1-7); 70 kD nRNP is described by Spritz, et al., Nucleic Acids Res. 24:10373-10391 (1987) (page 19, lines 3-5); A peptide of nRNP Sillekens, et al., EMBO J. 6:3841-3848 (1987) (page 20, lines 1-2); C peptide of nRNP, Sillekens, et al., Nucleic Acids Res. 25:8307-8321 (1988) (page 20, lines 9-10). Accordingly, correcting the typographical errors in the specification to match the published sequences, all of which are described by reference to the publications and incorporated by reference, does not introduce new matter.

U.S. SERIAL NO: 07/867, 819
FILED: April 13, 1992
AMENDMENT

Allowance of all claims 1-5 and 10-16, as amended, is earnestly solicited.

Respectfully submitted,

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Marked Up Version of Amended Specification Paragraphs

Pursuant to 37 C.F.R. § 1.121(b)(1)(iii)

Please replace page 4 of the specification with the following:

--Natl. Acad. Sci. USA. 76:5495 (1979) and Steitz, et al., Cold Spring Harbor Symposium Quant. Biol. 47:893 (1983), and may function as a termination factor for this enzyme, as reported by Gottlieb, et al., EMBO J. 8:841 (1989). A nucleic acid dependent ATPase/dATPase enzymatic activity has also been attributed to La/SSB by Bachmann, et al., Cell 60:85 (1990).

Anti-Sm antibodies are frequently associated with SLE. These autoantibodies precipitate snRNP containing the U1, U2, U4/U6 and U5 RNA. These complexes form the spliceosome and splice heterogenous nuclear RNA, as reported by Sharp, Science 235:766 (1987) and Maniatis and Reed, Nature 325:673 (1987). Anti-Sm antibodies are directed against one or a combination of six polypeptides: B (26 kDa), B' (27 kDa), D (13 kDa), E/F (11 kDa doublet) and G (less than 10 kDa).

Nearly all rheumatic disease patients who form an anti-Sm precipitin in Ouchterlony immunodiffusion have or eventually develop an anti-nRNP precipitin, as reported by Fisher, et al., Arthritis Rheum. 28:1348 (1985). Anti-Sm and anti-nRNP precipitins form a line of partial identify in Ouchterlony immunodiffusion, as discussed by Mattioli and Reichlin, J. Immunol. 110:1318 (1973). The basis for this partially shared antigenicity is explained by the composition of the U snRNP particles. The antigen for the anti-nRNP precipitin are the 70 kD, A, and C peptides that are unique to the U1 snRNP, B/B' and D peptides are also found on the U1 snRNP. The B/B' and D Ag, but not the 70 kDa, A or C, are found in the U2, U4/U6 and U5 snRNP. Hence, both anti-Sm and anti-nRNP bind anti-U1 snRNP activity, but only anti-Sm binds U2, U4/U6, and U5 snRNP.

U.S. Serial No. 07/648,205 filed January 31, 1991 by John B. Harley for "Assays and Treatments for Autoimmune Diseases", and U.S. Serial No. 07/472,947 entitled "Assays and Treatments for Autoimmune Diseases" filed January 31, 1990, now U.S. Patent No. 5,637,454, described a specific method to identify the etiologic or antigenic agent responsible for the production of autoantibodies characteristic of a particular disorder. The antigen is first --

Please replace page 24 of the specification with the following:

--After completion of an assay, pins were sonicated for 2 h in sonication buffer (40 g SDS, 4 ml ß-mercaptoethanol. and 62.4 g sodium phosphate to 4 liters) to remove antibodies, conjugate, and substrate. After sonication pins were washed twice in hot water and boiled in methanol for 2 min. Pins were then allowed to air dry for a minimum of 10 min and were stored with desiccant or used for another assay.

Epitope mapping of Sm B/B'. Peptides were first screened for reactivity with anti-human IgG conjugate alone. No background was demonstrated with anti-human IgG conjugate. Four normal human sera also showed minimal background reactivity with no specific antigenic regions demonstrated. SLE patients with autoimmune serology other than anti-Sm, anti-nRNP also showed no convincing, specific reactivity with any of the octapeptides. Other rheumatic serology tested included sera which formed precipitins with Ro/SSA as well as with both Ro/SSA and La/SSB Ag. Every patient who precipitated Sm and nRNP, however, showed considerable reactivity with various regions of the Sm B/B' protein. Ten Sm, nRNP sera were tested and all had a similar pattern of binding. Considerable reactivity was demonstrated in the proline-rich, carboxyl-terminal region of the protein. A repeated motif, PPPGMRPP (amino acids 4-11 of SEQ ID NO:14), is found in three regions of the Sm B/B' polypeptide and is similarly antigenic in each. In addition, a closely related fourth region, PPPGIRGP (amino acids 3-10 of SEQ ID NO:13), is bound by all the Sm, nRNP sera tested.

Several other antigenic regions were also detected in the first and middle portions of the polypeptide. These regions were not strongly reactive in all sera tested; however, they may be important by defining the differences in the very fine specificity between individuals with an autoimmune response to the B/B' Ag.

To elucidate which amino acids are essential for reactivity the average binding surrounding each purported epitope is presented along with the sequence of each relevant octapeptide in Figure 1. The octapeptide starting with amino acid 29,--

Substitution experiments of PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) *antigenic sequence.* Substitution of the arginine (R) with the other 19--

Please replace page 25 of the specification with the following:

--GTFKAFDK (SEQ ID NO:1), requires all eight residues for reactivity. The antigenicity of the reactive area in the region of octapeptide 45, however, appears to be based on a requirement for two lysines with an intercalated amino acid spacer. Binding is lost in octapeptides 44 and 53 when the two lysines with intercalated spacer is eliminated.

The sequence from octapeptides 140 to 145 all have moderately elevated average binding. Each shares a PQGR (amino acids 5-8 of SEQ ID NO:6) sequence that would appear to be critical for binding. The reactive site surrounding octapeptide 169, on the other hand, is not easily explained by a specific sequence. The repeated PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) and the similar PPPGIRGP (amino acids 3-10 of SEQ ID NO:13) found at octapeptides 191, 216, 223, and 231 share the hexamer, PPPGXR (SEQ ID NO:119), where X indicates an undetermined amino acid, in all of the octapeptides with the greatest binding. This repeated motif appears to be important in the binding of anti-Sm to linear epitopes of Sm B/B'.

Deletion experiments with PPPGMRPP (amino acids 4-11 of SEQ ID NO:14). Deletion experiments of the PPPGMRP (amino acids 4-10 of SEQ ID NO:14) sequence demonstrated that not all eight amino acids are required for antigenicity with the six patient sera tested, as shown in Figure 2a. Peptides were synthesized that deleted the carboxyl-terminal amino acid leaving a heptamer of PPPGMRP (amino acids 4-10 of SEQ ID NO:14), a hexamer of PPPGMR (amino acids 4-9 of SEQ ID NO:14), a pentamer of PPPGM (amino acids 4-8 of SEQ ID NO:14), and various tetrameres including PPPG (amino acids 4-7 of SEQ ID NO:14), PPGM (amino acids 5-8 of SEQ ID NO:14), PGMR (amino acids 6-9 of SEQ ID NO:14), GMRP (amino acids 7-10 of SEQ ID NO:14), and PPPP (amino acids 3-6 of SEQ ID NO:14). Binding appears to require at least the hexamer PPPGMR (amino acids 4-9 of SEQ ID NO:14)before the significant reactivity is lost. Greater than 60% of the reactivity to the PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) motif is destroyed when the sixth position arginine is removed. Removal of the carboxyl terminal prolines does not appear to significantly alter binding in the six patients tested. In addition no solely poly-proline sequence tested (PPPPP (SEQ ID NO:120), PPPP (amino acids 1-4 of SEQ ID NO:120), PPP, PP) has shown reactivity with these sera. Six of the 10 sera containing anti-Sm and anti-nRNP were tested with these deleted peptides and gave similar results. A normal serum did not bind any of these peptides.

Please replace page 26 of the specification with the following:

--naturally occurring amino acids demonstrated varying levels of reactivity with the six sera tested, as shown in Figure 2b. Substitution of the arginine by another positively charged amino acid, lysine (K), preserves more than 75% of the original reactivity. Another subset of the nine amino acids (containing F, G, H, I, S, T, V, W, and Y) retain approximately 50 to 65% of original binding, whereas the other nine amino acids reduce binding to less than 25%.

Synthesis of various similar epitopes. Short sequences of various proteins that displayed considerable homology with the PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) motif were also synthesized on polyethylene pins. These sequences and their reactivity, as measured by six different patient sera with anti-Sm and anti-nRNP precipitins from the first experiments. Octapeptides from nRNP A (23), nRNP C (24), and the EBV nuclear Ag-1 (25) were prepared. The nRNP A and one nRNP C sequence showed relatively no reactivity; however, the second nRNP C sequence (PAPGMRPP) (SEQ ID NO:116) showed over 90% of the original binding. In addition, the EBV sequence (which has five of six amino acid homology with the required antigenic portion of PPPGMRPP (amino acids 4-11 of SEQ ID NO:14)) is also significantly bound by Sm, nRNP precipitin positive patients.

All antigenic sites determined in this study require a positively charged amino acid. The size of these sites varies from two nonconsecutive basic amino acids to an entire octapeptide. The PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) motif appears to be the major linear antigenic epitope in all Sm, nRNP sera tested; however, this sequence does not appear to be bound by sera that contain an anti-Sm precipitin in the absence of an anti-nRNP precipitin. Sm precipitin alone patients bind two regions of Sm B/B'.

The carboxyl-terminal di-proline of the PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) motif is not required for antigenicity. Other deletion studies have also shown that the antigenicity of these autoantibodies are not specifically directed against the poly-proline regions. Peptides containing from two to six polyprolines have shown no reactivity with the sera used in this study. In addition, naturally--

Please replace page 27 of the specification with the following:

--occurring octapeptides of amino acid sequences 175-186 and 85-92 contain three or four consecutive prolines: none of these regions is antigenic. Also the very similar PPPGMIPP (SEQ ID NO:117) octapeptides does not bind to the sera precipitating Sm and nRNP.

Substitution experiments have shown that changing one amino acid can reduce reactivity of a sequence by more than 90%. Substituting the other 19 naturally occurring amino acids for the sixth amino acid, arginine (R), of the PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) sequence has divided these amino acids into three separate groups. Lysine (K), another basic amino acid, is the only substitution that preserves an average of more than 75% of the binding. With each of the sera tested, this amino acid substitution is consistently the most reactive. However, reactivity is ablated to less than 25% with nine amino acids. This set includes the acidic amino acids and their amines, the sulfur-containing amino acids, and the hydrophobic leucine, proline, and alanine. The other nine amino acids retain part, 42 to 65%, of the original binding. Phenylalanine was consistently the most reactive of this group. Nevertheless, the ability of lysine to substitute for arginine leads to the hypothesis that a positively charged amino acid at this position is preferred for the antigenicity of this sequence.

Example 4: Binding of monoclonal antibodies against peptide determinants of Sm B/B'.

Autoantibodies binding the Sm B and B' peptides (B/B') are commonly associated with systemic lupus erythematosus in man and in MRL *lpr/lpr* mice. KSm 3 and KSm 5 were derived from an unmanipulated MRL *lpr/lpr* mouse using hybridoma monoclonal antibody technology. Supernatants containing KSm 3 and KSm 5 monoclonal antibodies were collected from cloned murine hybridoma cell lines in RPMI 1640 with 10% fetal bovine serum, glutamine, penicillin and streptomycin. These monoclonal autoantibodies are both of the IgG2a subclass. The linear antigenic regions of these two anti-Sm B/B' murine monoclonal autoantibodies have been mapped using overlapping octapeptides. Unique epitopes are identified by each monoclonal. Monoclonal antibody KSm 5--

Please replace page 28 of the specification with the following:

--recognizes the peptide, PPPGMRPP (amino acids 4-11 of SEQ ID NO:14), which is repeated three times in the Sm B polypeptide. KSm 3 binds best to two very similar, almost neighboring octapeptides, PPPGIRGP (amino acids 3-10 of SEQ ID NO:13) and PGIRGPPP (SEQ ID NO:121). The two monoclonals do not crossreact. Both of these regions of Sm B/B' are major areas of antigenicity for human anti-Sm autoantibodies. Amino acid deletion and substitution in antigenic octapeptides show that binding to the KSm 5 epitope is lost with only slight modification. When the arginine in the sixth position PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) is substituted KSm 5 binding is found in an unexpected subset of octapeptides. Molecular dynamic modelling suggests that binding may be associated with a shared peptide backbone structure rather than charge or hydrophobicity of the substituted amino acid. In contrast, binding of KSm 3 to PPPGIRPP (SEQ ID NO:122) is abolished when the sixth position arginine is substituted by any other amino acid. These two murine autoantibodies bind distinct linear epitopes of Sm B/B' which are also bound by human anti-Sm B/B' autoantibodies. The bound epitopes are proline rich and contain an arginine in the fourth or sixth position. Thus, substitution at arginine and modelling experiments suggest very different mechanisms of binding for KSm 3 and KSm 5. A particular peptide backbone conformation, conferred by some amino acid sidechains at Position 6 of the octapeptide PPPGMRPP (amino acids 4-11 of SEQ ID NO:14), may be involved in KSm 5 binding while KSm 3 binding requires the specificity of the arginine side chain. Naturally arising autoantibodies may bind quite different features of similar peptide structures.

Substitution of either the fifth or sixth position amino acids of PPPGIRGP (amino acids 3-10 of SEQ ID NO:13) with all of the other nineteen naturally occurring amino acids shows unique patterns of reactivity. Substitution of the sixth amino acid arginine (R) shows that no other amino acid allows any degree of binding. When the fifth position isoleucine (I) is substituted, six of the twenty amino acids, phenylalanine (F) , histidine (E) threonine (T), valine (V) and tyrosine (Y), allow over 50% of the binding shown with the original octapeptide. Of these, thrconine has more reactivity than the original octapeptide.--

Please replace page 29 of the specification with the following:

--To determine structural motif common to peptides of either antigenic group, molecular dynamic simulations were performed for selected peptides which are antigenic to KSm 5 or KSm 3. A total of 2500 structures representing a nanosecond trajectory in time were accumulated for each peptide, and analyzed by monitoring backbone dihedral angles. At least one prevalent conformer could be chosen for all peptides as observed by a stable set of backbone dihedral angles. The root mean square difference between the KSm 3 positive peptides PPPGIRGP (amino acids 3-10 of SEQ ID NO:13) and PGIRGPPP (SEQ ID NO:121) of 4.87 A for the backbone atoms of eight residues indicates that there is no common backbone between these two peptides.

In contrast, the binding of KSm 5 to peptides substituted for arginine (R) in the sixth position in PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) suggest quite different binding requirements. Amino acids with side chains, $-(CH_3)_3$-NH-C-$NHNH_2$, $-CH_3$, -OH, and -H, all were roughly-equivalent in their ability to be bound by KSm 5. No feature of charge or hydrophobicity is shared by these amino acids. Also, lysine and histidine, the amino acids usually considered most similar to arginine, could not substitute and preserve binding. The possibility of a significant backbone conformation common to the native peptide PPPGMRPP (amino acids 4-11 of SEQ ID NO:14) off set by one or more amino acids in either direction was ruled out using OVRLAP18. The best match was found for the first few residues of the backbone with no offset. Accordingly, the conformation may play a role in binding.

Modifications and variations of the method and reagents of the present invention will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims--